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THE GREEN HYDRA SYMBIOSIS: ANALYSIS OF A FIELD POPULATION

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ABSTRACT

Green hydra were abundant on the alga *Vaucheria taylorii* in a shallow woodland stream near Lincoln, Nebraska, from March to June 1985. Green hydra were also found in low numbers throughout the winter. The algal-animal biomass characteristics of field populations of green hydra are compared to those of cultures established from the field populations and maintained under defined laboratory conditions. Although of similar protein biomass, freshly collected hydra contained greater numbers of symbiotic algae than did cultured hydra. Algae in field hydra were larger and contained more chlorophyll than algae in cultured hydra. Field populations of green hydra were highly productive; 16 $\mu g\ C \cdot h^{-1} \cdot mg$ hydra $protein^{-1}$ were fixed by the endosymbiotic algae at an irradiance of 28 $\mu E \cdot m^{-2} \cdot s^{-1}$.

INTRODUCTION

The green hydra-*Chlorella* association has been intensively studied for two decades. The association involves *Chlorella*-like algae living within the digestive cells of the freshwater polyp *Hydra viridissima*. It is one of a group of algal-invertebrate associations called phycozoans (Pardy, 1983) to emphasize their algal-animal composite nature. Much is known about the metabolic interdependency of the bionts, the recognition processes whereby hydra acquire algae, and some of the regulatory processes which stabilize the association. Comprehensive reviews concerning the association may be found in Cook (1980, 1981, 1983).

The majority of experimental studies have used laboratory cultures of green hydra. These hydra have been cultured under defined conditions with respect to temperature, light intensity, and feeding and maintenance schedules. Such stringent culture methods yield populations of green hydra of uniform size and age distributions, with stable densities of symbiotic algae. Although these laboratory cultures of green hydra are useful for certain experimental studies, they may bear little resemblance to green hydra living in ponds and streams. While a variety of ecological studies (Welch and Loomis, 1924; Miller, 1936; Bryden, 1952; Carrick, 1956; Cuker and Mozley, 1981; Ribi *et al.*, 1985) have attempted to describe the seasonal distribution and abundance of non-symbiotic hydra, similar studies of green hydra are lacking. Although green hydra have been collected from a variety of habitats [standing water (Whitney, 1907; Lashley, 1915), a swamp (Carrick, 1956), a river (McAuley, 1984), and roadside ditches (Forrest, 1959)], none of these studies have described the algal-animal biomass characteristics of freshly collected hydra.

In this paper we analyze the biomass parameters of symbiotic algae in field populations of green hydra, and compare these parameters to those of cultures established

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from the field populations and maintained under defined laboratory conditions. We also describe the seasonal variation in algal-animal biomass parameters of green hydra, and provide some estimates of the productivity of field populations. The results show that field populations of green hydra contain greater numbers of algae and are far more productive than hitherto measured in laboratory-cultured green hydra.

Materials and Methods

Location of study

A small stream near a large reservoir (Pawnee Reservoir) 10 miles northwest of Lincoln, Nebraska (40°50′37″N, 96°51′37″W) was chosen as a study site, as green hydra were known to occur in this stream (Pardy and Glider, 1984). The spring-fed stream (Pawnee stream) extended 1.5 km above the study site. There were two small ponds upstream from the site, one 122 m long and the other 50 m long. At the study site the water depth varied from 15 cm to 60 cm, depending on local rainfall. The bottom was muddy and often filled with litter from overhanging trees.

Collection and maintenance of hydra

Leaf litter and attached submerged vegetation were collected from the bottom of Pawnee stream; floating *Lemna* sp. plants were collected when present. As hydra were initially found in great abundance on algal mats of the filamentous chrysophyte *Vaucheria taylorii*, these mats were collected on a regular basis and examined for green hydra. Hydra were routinely sampled by removing four to five mats, about 200 cm^2 each, of *Vaucheria taylorii* and placing these into separate containers. In the laboratory, green hydra detached from the algal filaments and were readily collected with a Pasteur pipette. All analyses on hydra were performed within 24 h after collection.

To compare the biomass parameters of freshly collected hydra (=field hydra) with those of hydra maintained under defined conditions in the laboratory, hydra collected in March 1985 were brought into culture. These hydra were slowly acclimated to M solution (Muscatine and Lenhoff, 1965) by gradually replacing streamwater with M solution. Cultures were then maintained under continuous light at two irradiances (5 and 30 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 21°C and fed three times each week with freshly hatched *Artemia* nauplii. The culture medium was replaced daily; twice daily on feeding days. After 15 months of laboratory culture, population growth rates of Pawnee hydra maintained at 5 and 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ were measured as described by Muller-Parker and Pardy (1987). Population growth rate constants and doubling times were calculated according to Loomis (1954).

Ultrastructure of symbiotic algae

Transmission electron microscopy was used to examine the ultrastructure of symbiotic algae in freshly collected hydra. Hydra collected on March 18, 1985 were cut into pieces in phosphate buffered (0.1 *M*, pH 6.8) 3% glutaraldehyde at room temperature. The segments were fixed for 2 h in the glutaraldehyde fixative, then post-fixed with 1% OsO_4 in buffer for 1 h. Following dehydration in a graded series of ethanol, specimens were embedded in Epon 812. Sections stained in 2% aqueous uranyl acetate and lead citrate were viewed in a Philips 201 electron microscope operated at 60 kv.

Hydra biomass parameters

Algal and animal biomass parameters (protein, number of algae per μg hydra protein, number of algae per hydra digestive cell, algal chlorophyll and algal cell volumes) were measured on pooled samples of 100 hydra using previously described procedures (Muller-Parker and Pardy, 1987), with the exception that from 5 to 25 hydra were pooled to measure the number of algae per digestive cell. Biomass parameters of laboratory cultures of Pawnee hydra were measured after 15 months of culture. These hydra were last fed 48 hours before analysis.

Productivity of field hydra

The productivity of Pawnee hydra collected on April 3 and June 3, 1985 was measured in the laboratory. Carbon fixation at various irradiances was measured 24 h after collection. Groups of 25 hydra, each with one bud for uniformity, were incubated with $NaH^{14}CO_3$ (0.8 $\mu Ci \cdot ml^{-1}$) in 5.0 ml of filtered (>0.45 μm) streamwater supplemented with 5 m*M* $NaHCO_3$ in glass beakers covered with various layers of screen. Replicate groups of hydra were incubated in the dark to correct for any dark fixation of $NaH^{14}CO_3$. Incubation media were sampled at the start of the experiments for total ^{14}C activity. At the end of the one-hour incubations hydra were thoroughly rinsed in cold, filtered streamwater and then homogenized in distilled water. Organic ^{14}C retained by hydra, protein biomass, and numbers of algae were determined as previously described (Muller-Parker and Pardy, 1987). Total CO_2 in filtered streamwater was calculated from the total alkalinity–measured potentiometrically (Golterman, 1969)—of samples of filtered streamwater collected on July 17, 1986. After correction for dark fixation, the amounts of ^{14}C retained by hydra tissues were converted to rates of carbon fixation (Vollenweider, 1969) normalized to hydra protein biomass and to numbers of algae.

Results

Distribution of hydra in Pawnee stream

The distribution of green hydra in Pawnee stream from March 1985 to May 1986 was highly variable and appeared related to the presence of the filamentous chrysophyte alga, *Vaucheria taylorii* (Blum, 1971). Green hydra on *V. taylorii* were not immediately obvious, as the slender polyps and color of hydra bore remarkable resemblance to the algal filaments. Other samples of submerged vegetation yielded few hydra although individuals were occasionally found anchored to *Lemna* leaves as found by Pardy and Glider (1984).

Specimens of *Vaucheria taylorii* were identified by the characteristic structure of the antheridia and oogonia (Blum, 1971). These reproductive structures developed frequently in algae maintained in the laboratory at 21°C under continuous light. Thus it was possible to verify the taxonomic identity of this alga from various collections made throughout the year. This alga (formerly named *Vaucheria geminata* var. *racemosa*) has been reported from creeks around Lincoln, Nebraska (Saunders, 1894). *V. taylorii* was common in shallow, unshaded areas of the stream where there was slow water flow. The depth of the water above algal mats varied from 1 to 12 cm.

Peak abundances of field hydra averaged about 500 hydra per 200 cm^2 mat of *V. taylorii*, and occurred from March to June 1985. From June to September 1985 the stream occasionally dried up and did not contain aquatic vegetation or hydra. The site was not visited until January 1986, at which time *V. taylorii* was abundant and

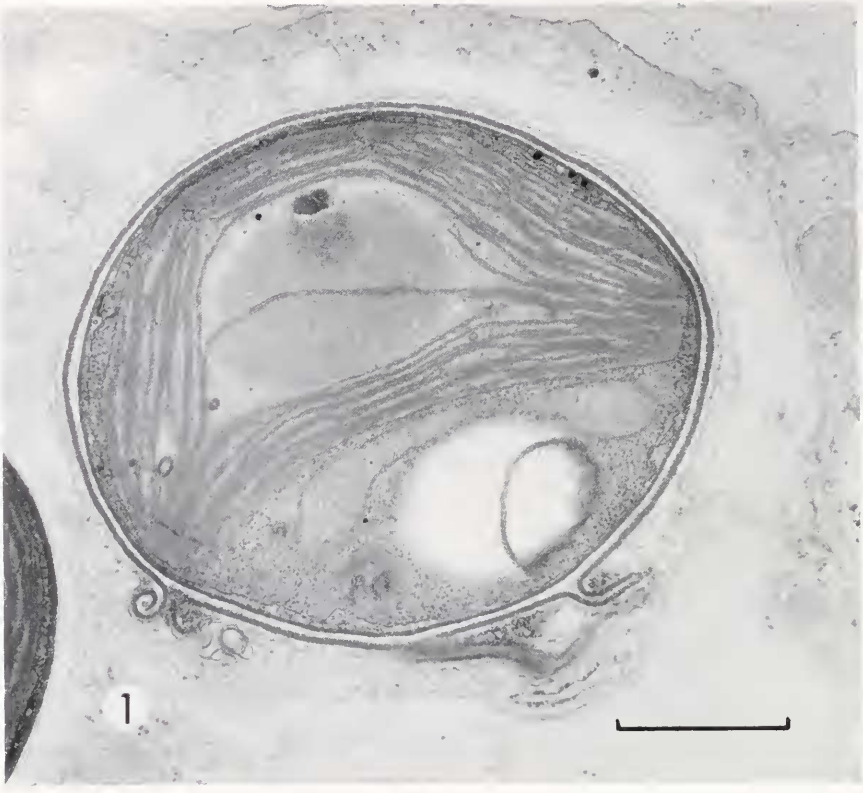

FIGURE 1. Algal symbiont in green hydra freshly collected from Pawnee stream. Scale bar = 1 μm.

hydra were found in low densities (1–10 individuals per 200 cm^2 mat). Hydra persisted on *V. taylorii* throughout the winter and spring of 1986. At all times, algal mats contained large numbers of zooplankton and the hydra were frequently observed to feed on these. Large nonsymbiotic brown hydra were often found among the green hydra, but never in great abundance.

All green hydra collected in March and April 1985 were asexual. Many had from one to five buds per individual. By early May, half of the hydra were sexual, bearing both ovaries and testes. Ninety-five percent of the collected hydra had gonads in late May. In early June the number of hydra bearing gonads had decreased to 50%; most of these bore testes only. Embryonic thecae resembled those described by McAuley (1984) for *Hydra viridissima*. A two-chambered theca, characteristic of *Chlorohydra hadleyi* (Forrest, 1959), was never seen. Released eggs did not develop under laboratory conditions.

Morphology, size, and chlorophyll content of algal symbionts from green hydra

The algal symbiont of hydra collected from Pawnee stream resembles the algae found in the English strain (Pardy, 1976; Jolley and Smith, 1978) of green hydra in that it possesses a pyrenoid traversed by a single thylakoid (Fig. 1).

The size of algae in field and cultured populations of green hydra is given in Table

TABLE I

Size of symbiotic algae in green hydra either (a) freshly collected or (b) maintained in culture for 15 months

	Algal diameter (μm)	Algal cell volume (μm^3)
(a) March 17, 1986	4.38 (±0.93)[a]	44
May 7, 1986	3.44 (±0.85)	21
(b) Low light (5 $\mu E \cdot m^{-2} \cdot s^{-1}$)	2.90 (±0.55)	13
High light (30 $\mu E \cdot m^{-2} \cdot s^{-1}$)	2.59 (±0.41)	9

[a] ±SD; n = 100.

I. Algae varied greatly in size in hydra collected on two dates in 1986, but cell volumes of algae in freshly collected hydra were at least twice those of algae in cultured green hydra (Table I).

Algae in field hydra contained more than four times the amount of chlorophyll measured in algae from cultured hydra (Table II). Although the chlorophyll content of algae varied greatly between cultured and field hydra, the ratio of chlorophyll *a* to chlorophyll *b* was about one in algae obtained from all hydra (Table II).

Algal-animal biomass parameters of green hydra

Algal numbers in hydra are readily obtained by counting the number of algae in dissociated digestive cells. Figure 2 shows the variation in numbers of algae in digestive cells in hydra collected in 1985 and 1986 from Pawnee stream. The average number of algae per cell ranged from 14 (March 1986) to 34 (June 1985). Ambient stream temperatures are also given in Figure 2. There appears to be no relationship between algal densities in green hydra and water temperature. Hydra collected in near-freezing waters (January and February 1986) under substantial layers of ice and

TABLE II

Chlorophyll content of algae isolated from green hydra either (a) freshly collected in 1985 or (b) maintained in culture for 15 months

	Total Chl·cell^{-1} (pg)	Chl *a*·cell^{-1} (pg)	Chl *b*·cell^{-1} (pg)	*a*/*b*
(a) March 18	0.63	0.32	0.31	1.01
March 20	0.80	0.44	0.36	1.20
April 3	0.68	0.35	0.33	1.06
May 7	0.77	0.40	0.37	1.05
May 22	0.45	0.21	0.23	0.92
June 3	0.49	0.28	0.21	1.34
(b) Low light (5 $\mu E \cdot m^{-2} \cdot s^{-1}$)	0.15 (±.02)[a]	0.08 (±.01)	0.07 (±.01)	1.14 (±.06)
High light (30 $\mu E \cdot m^{-2} \cdot s^{-1}$)	0.04 (±.01)	0.02 (±.004)	0.02 (±.006)	1.16 (±.24)

[a] ±SD; n = 5.

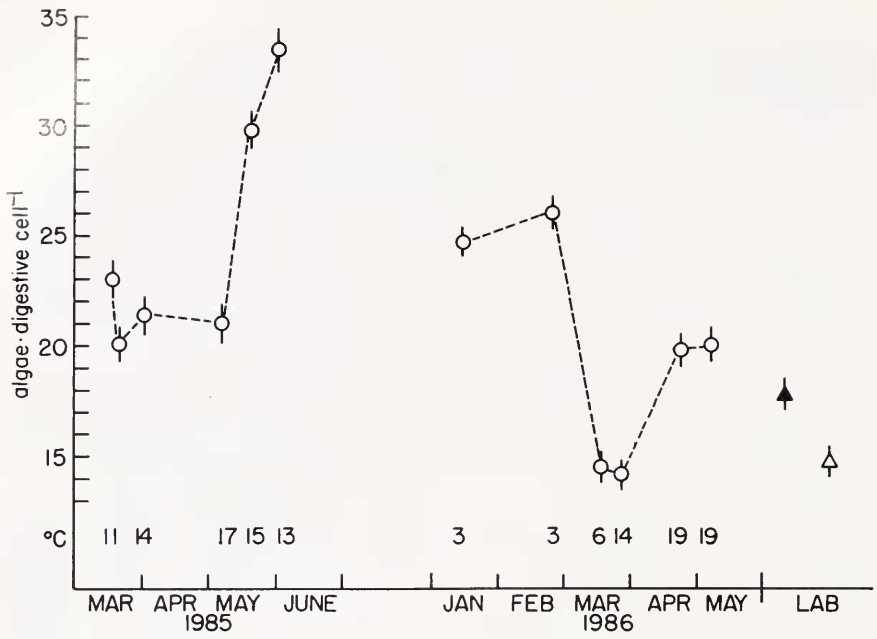

FIGURE 2. Number of algae per digestive cell (open circles, ± SE) in hydra collected from Pawnee stream in 1985 and 1986. Ambient streamwater temperatures measured on the collection dates are included near the origin of the y-axis. For comparison, the mean numbers of algae per digestive cell of cultured Pawnee hydra maintained at 5 $\mu E \cdot m^{-2} \cdot s^{-1}$ (solid triangles) and at 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ (open triangles) are included.

snow contained a full complement of algae (Fig. 2). The numbers of algae per digestive cell of cultured hydra are included in Figure 2 for comparison. Digestive cells of cultured hydra contained under 20 algae, whereas 50 or more algae per digestive cell were frequently counted in freshly collected green hydra. In general, digestive cells of field populations of these green hydra contained greater numbers of symbiotic algae than those of hydra maintained in laboratory culture.

The density of symbiotic algae in freshly collected hydra was also measured by determining the number of algae per μg hydra protein in 1985 (Fig. 3a). Algal densities varied two-fold over a four month period; algal densities measured in June were double those obtained in March. This change in algal density coincided with a shift from a predominantly asexual population to a predominantly sexual population. Algal densities in cultured hydra were within the range obtained for field hydra; hydra maintained at a low irradiance contained more algae than those maintained at a high irradiance (Fig. 3a).

The size of field hydra was estimated by measuring the weight of protein per hydra individual. Figure 3b shows that the protein content of field hydra ranged from 2 to 8 μg protein, and that the increase in algal density (Fig. 3a) was accompanied by a decrease in protein content. Thus, hydra collected in June 1985 were smaller and contained higher densities of algae than those collected in March 1985.

Laboratory cultures of Pawnee hydra contained high protein biomass and low algal densities in comparison to field populations (Fig. 3a, b). Hydra maintained at 5 $\mu E \cdot m^{-2} \cdot s^{-1}$ were larger and contained more algae than individuals maintained at 30

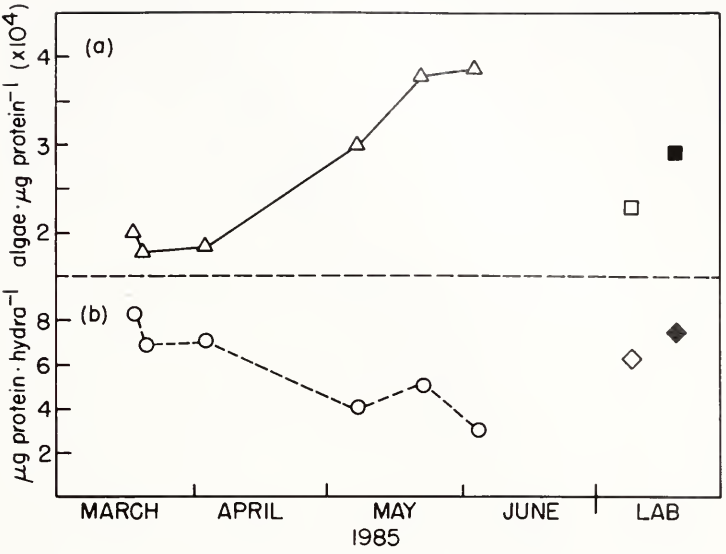

FIGURE 3. (a) Number of algae per μg hydra protein in hydra collected from Pawnee stream in 1985 (open triangles). (b) Protein biomass of hydra collected from Pawnee stream in 1985 (open circles). Parameters obtained in cultured hydra (Lab) maintained at 5 $\mu E \cdot m^{-2} \cdot s^{-1}$ (solid symbols) and at 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ (open symbols) are included to the right of the field data.

$\mu E \cdot m^{-2} \cdot s^{-1}$. Population growth rate constants of Pawnee hydra maintained at both irradiance levels were the same and averaged 0.28 day^{-1}, which resulted in a doubling time of 2.5 days.

Productivity of field hydra

The productivity of green hydra collected from Pawnee stream on April 3, 1985 and on June 3, 1985 was measured in the laboratory. The amount of carbon fixed by these hydra increased linearly with increase in irradiance from 0 to 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 4). Photosynthetic efficiencies (the slopes of the lines in Fig. 4) were normalized to protein biomass and number of algae. Photosynthetic efficiencies of April hydra were 0.45 $\mu g\ C \cdot (\mu E \cdot m^{-2} \cdot s^{-1})^{-1} \cdot h^{-1} \cdot mg^{-1}$ protein and 0.022 $\mu g\ C \cdot (\mu E \cdot m^{-2} \cdot s^{-1})^{-1} \cdot h^{-1} \cdot 10^6$ $algae^{-1}$, whereas those of June hydra were 0.60 $\mu g\ C \cdot (\mu E \cdot m^{-2} \cdot s^{-1})^{-1} \cdot h^{-1} \cdot mg^{-1}$ protein and 0.016 $\mu g\ C \cdot (\mu E \cdot m^{-2} \cdot s^{-1})^{-1} \cdot h^{-1} \cdot 10^6$ $algae^{-1}$. Hydra collected on these two dates differed greatly in algal density (Fig. 3a) and in protein biomass (Fig. 3b). The hydra collected on April 3 contained twice the amount of protein and half the number of algae as hydra collected on June 3, 1985. Figure 4a shows that on a protein basis the smaller hydra with greater numbers of algae (June hydra) were more productive than the April hydra. When the data were normalized to numbers of algae, hydra with low densities (April hydra) were more productive on a cell basis (Fig. 4b). These results suggest that although high algal densities result in greater productivity on a unit biomass basis, the amount of carbon fixed per alga is greatly reduced.

DISCUSSION

We have shown that field populations of symbiotic hydra are highly productive and maintain high densities of algae throughout the year. There may be significant

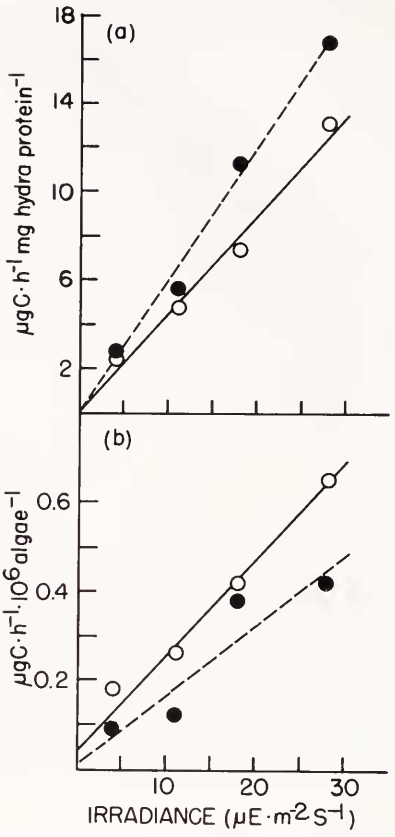

FIGURE 4. Productivity of field hydra at different irradiances. Rates were normalized to (a) protein biomass and (b) numbers of algae. Open circles represent production rates of hydra in April 1985 and closed circles are those obtained for hydra in June 1985.

differences in the algal and animal biomass parameters and productivity of field and laboratory populations of green hydra. Symbiotic algae isolated from field populations of green hydra are larger and contain more chlorophyll than those isolated from cultured hydra. When maintained under controlled laboratory conditions, the algal-animal biomass parameters and growth rates of these hydra resemble those of the Florida hydra strain (Muller-Parker and Pardy, 1987) kept under the same conditions.

Algal-animal biomass parameters

The biomass parameters of green hydra collected in 1985 and 1986 from one site were quite variable. Variation in protein biomass of hydra, in numbers of algae, and size and chlorophyll content of the algae can result from several processes which are not mutually exclusive. Changes in the physical environment (light, temperature, water flow) and in prey availability may affect these parameters. Genetic differences in hydra populations collected at different times may be important (McAuley, 1984). The relative influence of these factors cannot be presently assessed.

Hydra were found in Pawnee stream throughout the winter. Others have found both symbiotic hydra (Whitney, 1907) and non-symbiotic hydra (Carrick, 1956) in water of very low temperatures. However this is the first report which shows that green hydra maintain high densities of symbiotic algae throughout the winter.

There was a great increase in algal density and decrease in protein biomass of field hydra during the spring of 1985 (Fig. 3a, b). At the same time these hydra shifted from a predominantly asexual population to a sexual population. This suggests that sexual individuals may contain higher densities of algae than non-sexual hydra. This needs to be confirmed in hydra maintained under controlled conditions in the laboratory.

The most striking difference between the algal-animal biomass parameters of freshly collected and cultured Pawnee hydra was the large difference in algal cell volume (Table I) and chlorophyll content of the algae (Table II). The large size of the algae and the great number of algae per digestive cell (Fig. 2) in freshly collected hydra show that these hydra contain a proportionately greater ratio of plant to animal biomass than cultured hydra. This suggests that regulatory processes governing the number of algae in hydra cells may be substantially different under field conditions.

Hydra collected from Pawnee stream and maintained in culture for 15 months showed similar responses to light as the Florida strain of hydra maintained under the same conditions (Muller-Parker and Pardy, 1987). In both Pawnee and Florida hydra, protein biomass and algal densities decreased with increase in culture irradiance, whereas population growth rates were unaffected by irradiance. An increase in algal cell volume and decrease in chlorophyll per alga occurred in both Pawnee and Florida hydra with increase in culture irradiance. These results suggest that green hydra from different localities respond in a consistent manner to changes in culture irradiance.

Pawnee hydra were smaller than Florida hydra as the protein biomass of cultured Pawnee hydra maintained at 5 and 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ (7.6 and 6.3 μg) was less than that of Florida hydra maintained at the same irradiances (11.0 and 9.5 μg; Muller-Parker and Pardy, 1987). The population growth rates of Pawnee hydra were slightly higher than those of Florida hydra; population doubling times for Pawnee hydra were 2.5 days whereas those of Florida hydra averaged about 3 days. Algal cell volume and the number of algae per digestive cell were similar for both Pawnee and Florida hydra.

Productivity of field collected Pawnee hydra

The productivity of hydra collected from Pawnee stream on two dates was high; rates of carbon fixation were over four times as great as those obtained with cultured Florida hydra incubated under similar conditions (Muller-Parker and Pardy, 1987). Although field Pawnee hydra and cultured Florida hydra are not strictly comparable, the productivity of cultured Pawnee hydra was not measured in this study. The previous light history may affect photosynthetic rates, as photosynthetic efficiencies derived for Pawnee hydra were greater than those obtained for cultured Florida hydra (Muller-Parker and Pardy, 1987). Differences in productivity cannot be attributed to differences in protein biomass and density of algae of field and laboratory hydra, since Pawnee hydra collected in April and June 1985 were substantially different in these two parameters. However, algal cell size and chlorophyll content of algae were much greater in freshly collected Pawnee hydra than in cultured Florida hydra, which may account for the high productivity of Pawnee hydra.

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